

Page 10, third full paragraph, which wraps to page 11:

B₂ In a first set of optimization experiments, efficient incorporation of pal-prot A was documented in four cell lines (Fig. 1A) as detected with FITC-conjugated human IgG. As a negative control, nonderivatized protein A lacked the capacity to bind to the same cells. Data from the FACStar[®] flow cytometer analysis was plotted as arbitrary units of log₁₀ fluorescence intensity versus number of EL-4 cells; membrane incorporation was dose dependent and started to plateau at about 33 µg/ml pal-prot A, as shown in Fig. 1B. EL-4 cells were incubated with 33 µg/ml pal-prot A for the indicated periods of time and processed as above; pal-prot A incorporation was rapid, appearing immediately after addition to the cells and reaching a plateau at ~1 h, as shown in Fig. 1C. This data demonstrates that numerous different cell lines can be used in the present protein transfer methods, and that the lipidated protein was incorporated into the cell fairly rapidly.

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B₃ Cells precoated with pal-prot A were washed once and resuspended in RPMI 1640 medium ($3-7 \times 10^6$ cells/ml). pREP7B-transfected K562 cells (K562/REP7b) were serially incubated with 33 µg/ml protein A for 2 h, 33 µg/ml Fcγ₁ fusion protein for 1 h, and BB-1 as primary Ab and FITC-conjugated goat anti-mouse IgG as secondary Ab. To monitor protein delivery, 10^6 cells were washed twice with the same buffer as above, incubated on ice for 1 h with 1 µg of human B7-specific mAb BB-1 (PharMingen, San Diego, CA) in 100 µl of buffer. Cells were washed once and immunostained (on ice for 1 h) with 100 µl of 1:100 diluted FITC-conjugated goat F(ab')₂ anti-mouse Ig (Boehringer Mannheim, Indianapolis, IN) as secondary Ab. Cells were washed once, resuspended in PBS, and analyzed on a FACStar[®] flow cytometer.

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B₄ PBMC were isolated from fresh whole blood by Ficoll density centrifugation. T-cells were purified by two rounds of treatment with Lympho-kwik (One Lambda, Canoga Park, CA). T-cell purity was verified by lack of a proliferative response to phytohemagglutinin ("PHA") or PMA in the absence of accessory cells. The human CD3-specific mAb HIT3a (PharMingen) was bound to 96-well plates at the indicated concentrations and used in this form to provide a first activating signal to T-cells. Alternatively, PHA was used in soluble form as a source of a first signal. K562 cells transfected with the negative control vector pREP7β (K562/pREP7β) were precoated with